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Acidosis and hypoxic medullary injury in the isolated perfused kidney

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Acidosis and hypoxic medullary injury in the isolated perfused kidney. The effects of acidosis on renal function and morphology were examined in the isolated perfused rat kidney (IPK). Kidneys were perfused with oxygenated Krebs-Henseleit-albumin medium for 60 minutes at pH 7.4 or pH 7.0. At the lower pH, GFR was reduced by 25%, TR_{Na} by 32% and oxygen consumption by 41% as compared to perfusion at pH 7.4 (all $P < 0.05$). In addition, the usual hypoxic injury observed in the medullary thick ascending limb of the Loop of Henle (TAL) in the IPK at pH 7.4 (consisting of nuclear pyknosis and focal fragmentation necrosis) was reduced by acidosis from 62% to 14% of tubules involved ($P < 0.005$). This cytoprotection was not the result of improved oxygenation since O_2 delivery was actually slightly reduced at pH 7.0 compared to pH 7.4. Furthermore, acidosis was protective even after perfusion with non-oxygenated media (42% tubules damaged at pH 7.0 vs. 95% of tubules damaged at pH 7.4; $P < 0.01$), making it very unlikely that the effect of acidosis is to improve TAL oxygenation. Since previous studies indicate that the TAL lesion is transport dependent and prevented in the non-filtering kidney, it was possible that the decrease in GFR associated with acidosis could account for decreased injury. The GFR was manipulated by alterations in perfusion pressure or albumin concentration, and no consistent relationship between the extent of injury and GFR could be shown at either pH over a wide range of GFR values. Therefore, acidosis protected the TAL from hypoxic injury by a mechanism apparently independent of oxygen or solute delivery. That this is probably a direct cytoprotective effect was further supported by the finding that acidosis decreased TAL damage induced by amphotericin, a polyene ionophore previously shown to exacerbate cellular hypoxia apparently by stimulating transport activity. The effects of acidosis in the kidney are thus complex and include both a depression of physiologic function and protection from hypoxic necrosis. The mechanism of this cytoprotection remains unknown.

Acidosis occurs in most situations in which there is hypoxia or ischemia, but its role in the injury process is incompletely understood. Acidosis itself can cause significant functional impairment [1, 2]. However, cell integrity in terms of morphologic damage or ability to recover from the insult seems to be protected when acidosis accompanies O_2 deprivation [3–8]. It is possible that there is a trade-off of depressed function during the insult for preservation of cell viability. Such a hypothesis would be consistent with the concept that the metabolic demand imposed by cell work contributes importantly to hypoxic injury [9]. To test this idea studies were undertaken to determine the effects of acidosis in the isolated perfused kidney. In the hemoglobin-free perfused kidney necrosis consistently develops in the thick ascending limb of the loop of Henle (TAL) [10]. Previous studies have determined that this lesion is caused by hypoxia related to the low O_2 carrying capacity of the perfusate [11] and to O_2 demand related to transport activity [12, 13]. In the present study the effect of lowering the pH of the perfusion media on the development of this lesion and on the general physiologic function of the kidney were examined.

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Methods

Perfusion of isolated kidneys

Male Sprague-Dawley rats, weighing 310 to 465 g, fed Purina rat chow and allowed free access to water, were used for all experiments. Perfusion of the right kidney was performed by modification of the technique described by Ross et al [14]. In brief, the animals were anesthetized by intraperitoneal injection of pentobarbital (60 mg per kilogram). After a midline abdominal incision, a polyethylene catheter (PE-10) was inserted into the right ureter. A glass canula was inserted into the right renal artery via the superior mesentery artery and across the aorta. The continuously recirculated perfusate was Krebs-Henseleit bicarbonate buffer containing bovine serum albumin. The substrate was glucose at 5 mM. No amino acids were added to the perfusate. Perfusions lasted 60 minutes.

Clearance periods of 10 minutes each were observed from the start of perfusion for physiologic determinations. Optimum function was generally observed during the 20 to 30 minute period and therefore the data reported is for the 30 minute collection. Glomerular filtration rate (GFR) was estimated from the clearance of [^{14}C]inulin. Absolute sodium reabsorption (TR_{Na}) and fractional excretion of sodium (FE_{Na}) were calculated from urine and perfusate sodium concentrations (measured with an Instrument Lab Inc. #343 flame photometer, Lexington, Massachusetts, USA), urine flow and the inulin clearance. The perfusate flow rate was monitored by a precalibrated in-line flowmeter tube with a tantalum float (Thomas Scientific, Swedesboro, New Jersey, USA). Oxygen consumption was calculated from the arteriovenous difference in O_2 content of perfusate and the perfusion flow rate.

During perfusion the pH of the media was continuously monitored with a Beckman pH meter with the electrode im-

Table 1. Physiology of the isolated perfused kidney under various conditions

pH	Conditions	*N*	GFR	TR_{Na}	FE_{Na}	$Q \cdot O_2$	DO_2	U/O
7.4	Control	6	511 ± 38	68 ± 7	10.1 ± 2.3	4.33 ± 0.44	14.04 ± 0.88	62 ± 11
	N_2/CO_2	2	21 ± 18[a]	ND	ND	1.40 ± 0.06[a]	2.73 ± 0.50[a]	9 ± 1[a]
	Amphotericin	3	261 ± 178[a]	42 ± 36	28 ± 10	3.40 ± 0.19	8.40 ± 1.59[a]	72 ± 48
	High pressure	4	712 ± 144	92 ± 20	13.5 ± 2.7	4.58 ± 0.69	15.13 ± 1.78	118 ± 18[a]
	Hyperoncotic	3	131 ± 90[a]	47 ± 13	3.2 ± 1.7[a]	4.74 ± 0.80	16.07 ± 3.27	11 ± 9[a]
7.0	Control	5	384 ± 56[a]	46 ± 7[a]	15.8 ± 1.4[a]	2.54 ± 0.54[a]	12.29 ± 0.84	69 ± 16
	N_2/CO_2	3	135 ± 43[b]	ND	ND	2.16 ± 0.56	3.69 ± 0.66[b]	39 ± 7
	Amphotericin	4	489 ± 106	54 ± 13	23.9 ± 4.7[b]	4.26 ± 0.46[b]	8.30 ± 1.17[b]	141 ± 25[b]
	High pressure	5	428 ± 55	46 ± 6	23.5 ± 3.8[b]	3.58 ± 0.70	11.43 ± 1.35	128 ± 22[b]

Clearance data were collected at 30 min and O_2 data at 40 min during 60 min perfusions at pH 7.4 or pH 7.0. The *control* conditions were a perfusion pressure of 100 mm Hg and 6.7 g albumin/dl Krebs-Henseleit perfusion media gassed with 95% O_2/5% CO_2. *High pressure* perfusions were at 125-130 mm Hg and *Hyperoncotic* perfusions were with 8 g% albumin. *Amphotericin* was added at 5×10^{-5} M after 20 min of perfusion. In N_2/CO_2 perfusions media was gassed with 95% N_2/5% CO_2. *N* refers to the number of experiments under each condition. Glomerular filtration rate (GFR) and urine output (U/O) are in μl/min/g kidney wet weight. Tubular reabsorption of sodium (TR_{Na}) is in μEq/min/g kidney wet weight. Fractional excretion of sodium (FE_{Na}) is in percent. Oxygen consumption ($Q \cdot O_2$) and O_2 delivery (DO_2) are in μmol/min/g kidney wet weight. ND = not done.

[a] Significant difference from pH 7.4 control for experimental groups at pH 7.4 and for pH 7.0 control group.

[b] Significant difference from pH 7.0 control group for experimental groups at pH 7.0.

mersed in the perfusate reservoir (Beckman Instruments, Fullerton, California, USA). The readings closely corresponded to those in the arterial samples taken at 20, 40 and 60 minutes for pO_2 measurement where pH was simultaneously determined on a Corning 158 pH/blood gas analyzer (Corning, Midfield, Massachusetts, USA.

Morphologic studies

At the end of the perfusion period 1.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, was introduced into the perfusion arterial line via a three-way stopcock. Perfusion fixation of the kidney was carried out for 7 to 10 minutes. A 1 to 2 mm thick coronal slice from the midportion of the kidney was removed and a 4 × 8 mm section containing cortex, outer medulla and papilla was removed. This section was fixed for an additional 24 hours in glutaraldehyde and then removed to 0.15 M phosphate buffer, pH 7.3. The tissue was dehydrated in graded ethanol, then infiltrated and embedded in glycolmethacrylate [15]. One micron thick sections were stained with methylene blue.

For the quantitation of the injury in the TAL, the slide was placed on a mechanical stage and all TAL tubules in a 40× high-power field passing through the midportion of the inner stripe were evaluated for the presence or absence of the typical severe hypoxic lesion, consisting of nuclear pyknosis and apparent fragmentation of the cells with focal denudation of the basement membrane [10, 11]. The percentage of tubules with such changes was recorded. The morphologic evaluation was done on coded slides without knowledge of the experimental conditions.

For electron microscopy, 1 mm^3 tissue fragments were taken from the inner stripe after the perfusion fixation and were fixed for an additional 24 hours in glutaraldehyde. This was followed by dehydration and embedding in an Araldite-Embed 812 mixture by standard procedures, and thin sections were examined on a Phillips 201 transmission electron microscope.

Experimental groups

1. Regular (oxygenated) perfusions. The perfusion media was gassed with 95% O_2/5% CO_2. Albumin concentration was 6.7 g%. Perfusion pressure was 100 mm Hg at the tip of the cannula. pH was 7.4 throughout each experiment.

2. Acidotic perfusions. Perfusions were as in group 1 except that perfusion media pH was titrated to 7.0 by addition of 1 N HCl.

3. High pressure perfusions. Perfusions were at either pH 7.4 or pH 7.0 as in groups 1 and 2, except that perfusion pressure was 125 to 130 mm Hg at the cannula tip rather than the usual 100 mm Hg.

4. Hyperoncotic perfusions. Perfusions were as in group 1 except that albumin was 8 g% rather than the usual 6.7 g%.

5. Nonoxygenated perfusions. Perfusions were as in groups 1 or 2 except that the media was gassed with 95% N_2/5% CO_2.

6. Amphotericin perfusions. Perfusions were as in groups 1 or 2 except that 5×10^{-5} M amphotericin was added. The amphotericin was dissolved in acidic methanol at 5×10^{-3} M just prior to perfusion and diluted 1:100 into the perfusion media after 20 minutes of perfusion equilibration. It was found that the enhancement of injury and impairment of function caused by this agent were not observed if the stock solution was prepared hours in advance of perfusion emphasizing the importance of freshly preparing the drug for each experiment.

Statistical analysis

The data are expressed as mean ± SEM. Comparison between the groups was done using the unpaired Student *t*-test or the Mann-Whitney U-test, with statistically significant differences between groups presumed at $P < 0.05$ in a one-tailed analysis.

Results

Physiologic effects of acidosis in the oxygenated perfused kidney

Comparison of the function of the isolated perfused kidney at pH 7.4 and pH 7.0 under various conditions is shown in Table

1. In oxygenated perfusions acidosis caused a 25% decline in GFR, a 32% decline in tubular reabsorption of sodium, a 41% decline in oxygen consumption, and a 13% decline in oxygen delivery relative to values obtained at pH 7.4. There was no effect on urine output.

Effect of acidosis on tubule injury in the oxygenated perfused kidney

As previously reported [10–13], renal perfusion with hemoglobin free-oxygenated media consistently produces severe fragmentation injury in the TAL tubules (Fig. 1). At pH 7.4 the proportion of tubules with such damage after 60 minutes of oxygenated perfusion was 62% ± 7%. At pH 7.0, however, the percentage of tubules exhibiting these severe lesions was reduced to 14% ± 10% ($P < 0.005$; Fig. 2). After acidotic perfusion most of the tubules were either intact or exhibited only mitochondrial swelling (Fig. 1).

Effect of severe hypoxia

Gassing perfusion media with N_2/CO_2 rather than O_2/CO_2 markedly diminished GFR (Table 1) and increased TAL damage (Fig. 2). The extent of injury to TAL after N_2/CO_2 perfusion at pH 7.0 was significantly less than that at pH 7.4 (42 ± 9% vs. 95 ± 4%; $P < 0.01$).

In the N_2/CO_2 perfused kidneys proximal tubule injury identical to that previously described [16] was present at pH 7.4. The lesions consisted of brush border clubbing and high-grade mitochondrial swelling in S1 and S2, and of cell swelling or fragmentation necrosis in S3. At pH 7.0 the S1 and S2 injury appeared reduced in extent, while S3 tubule damage remained evident except for those tubules or portions of tubules adjacent to major vascular bundles.

Effect of amphotericin perfusion

Amphotericin has been shown to increase the extent of TAL necrosis in this model [17], and the present experiments showed that at pH 7.4 nearly all TAL were necrotic after amphotericin perfusion (Fig. 2). Acidosis decreased necrosis during amphotericin perfusion from 98 ± 1% of tubules involved to 67 ± 17% ($P < 0.05$). Other consistent effects of amphotericin were to decrease O_2 delivery to the kidney (Table 1) and to progressively depress glomerular filtration. By 30 minutes after addition of amphotericin GFR (in μl/min/g) was 15 ± 3 at pH 7.4 and 11 ± 5 at pH 7.0.

Effect of high perfusion pressure

Oxygenated perfusion at 125 to 130 mm Hg rather than the usual 100 mm Hg resulted in a 39% increase in GFR at pH 7.4 ($P = 0.05$) and a 12% increase in GFR at pH 7.0 (not significant). There was no effect on the extent of injury in the acidotic perfusion, while at pH 7.4 there was a trend toward decreased injury with very high GFR (Fig. 3). The extent of injury in the high perfusion pressure experiments at pH 7.4 inversely correlated with the O_2 delivery in each instance ($r = -0.985$).

Effect of hyperoncotic perfusion

Oxygenated perfusion at pH 7.4 with the perfusate albumin concentration at 8 g% rather than the usual 6.7 g% resulted in a 74% decrease in GFR relative to the control pH 7.4 value ($P < 0.0005$). Lowering the GFR by this amount at pH 7.4 was not sufficient to account for the cytoprotection afforded by acidosis, however, since severe injury was still present in 45 ± 1% of TAL tubules (Fig. 3).

Discussion

The effects of extracellular acidosis on aspects of the function and morphology in the isolated perfused rat kidney are described. Acidosis depressed glomerular filtration rate, sodium reabsorption, and oxygen consumption in the IPK, but at the same time afforded considerable protection against hypoxic TAL necrosis in a variety of situations. The results emphasize the potential interpretive problems which can occur in studies relying solely on either functional or morphologic parameters in the assessment of injury and protection from injury. The results also establish lowering of perfusate pH as an alternative maneuver (along with the previously described use of ouabain, furosemide, amino acids and hemoglobin [reviewed in reference 9]) for protecting against morphologic damage in this model of hypoxic injury.

The mechanism of the cytoprotective effect of acidosis remains unknown. Two possibilities have been examined in these experiments: first, that acidosis improves TAL oxygenation and second, that decreased solute delivery to the nephron secondary to the decrease in GFR accounts for the protective effect. The present results demonstrate that acidosis did not improve the perfusate flow or oxygen delivery to the kidney. Furthermore, since acidosis was protective even during nonoxygenated perfusion, any role for local enhancement of O_2 delivery in the protection of TAL integrity, such as might occur with intrarenal redistribution of flow to the medulla, appears to be ruled out.

The results also suggest that decreased solute delivery to the nephron during perfusion at pH 7.0 is not the critical protective factor. This possible mechanism was considered because it has been shown that the TAL lesion can be prevented in the non-filtering kidney [12]. Furthermore, the extent of a similar transport-dependent fragmentation necrosis in the S3 segment of the proximal tubule has been previously shown to directly correlate with glomerular filtration rate [18]. Therefore, it appeared possible that the effect of acidosis to decrease GFR (and thus indirectly to decrease transport activity in the nephron) could have accounted for the decrease in tubule necrosis. However, even when GFR was decreased by 75% during perfusion with moderately hyperoncotic media at pH 7.4, the degree of cytoprotection seen with a reduction in GFR of only 25% during acidotic perfusion was not observed. The decreased GFR observed at pH 7.0 therefore appeared insufficient in itself to account for the cytoprotection by acidosis. The lack of increased frequency of TAL injury in those acidotic kidneys whose GFR fell within the range of GFR seen during control pH 7.4 perfusions (Fig. 3) underscore the dissociation between acidotic cytoprotection and the relative GFR. The direct relationship between the extent of injury and GFR previously reported for S3 was not evident for TAL at either pH.

These results demonstrating that TAL protection by acidosis is independent of oxygen or solute delivery suggest that extracellular acidosis may have a direct cellular protective effect. This hypothesis is strengthened by the observed protection which acidosis afforded to the TAL during amphotericin perfusion. The amphotericin model is admittedly complex, but at pH

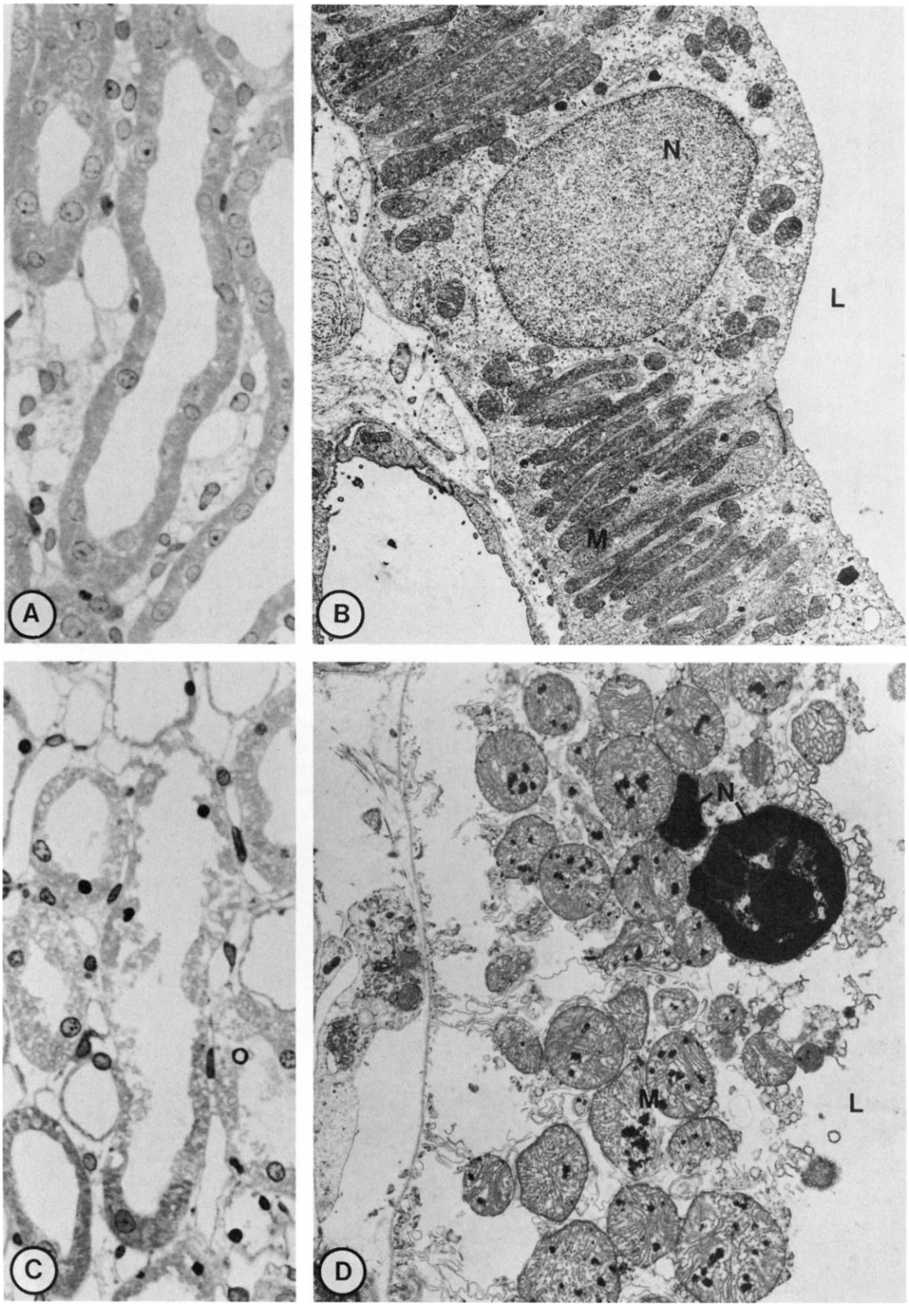

Fig. 1. *See caption next page.*

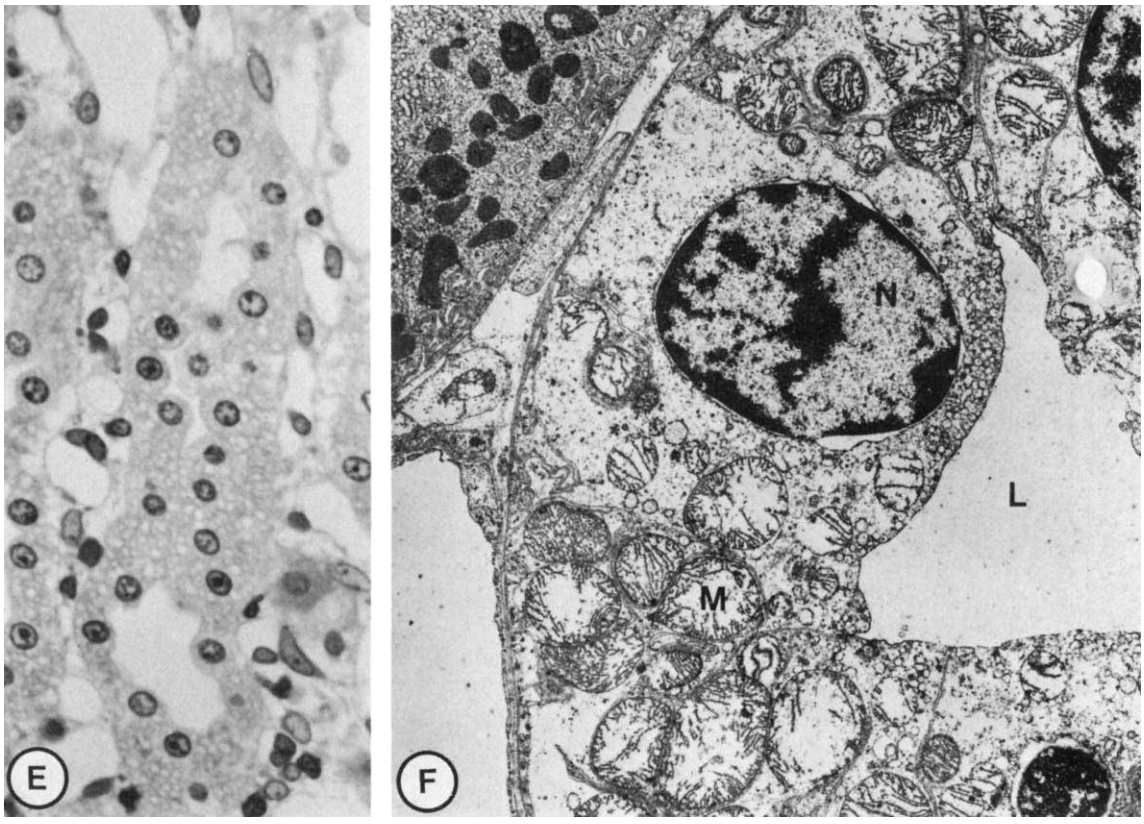

Fig. 1. *Effect of acidosis on TAL morphology in the perfused kidney.* The inner stripe of the outer medulla is shown after perfusion fixation of the kidney in an untreated control rat in vivo (**A** and **B**), a kidney perfused for 60 min at pH 7.4 (**C** and **D**) and a kidney perfused for 60 min at pH 7.0 (**E** and **F**). Note the extensive nuclear pyknosis, mitochondrial densities and membrane fragmentation after perfusion at pH 7.4. Such "fragmentation necrosis" is much reduced in extent at pH 7.0 where the predominant lesion is mitochondrial swelling. L = Lumen, M = Mitochondria, N = Nucleus. A, C and E at 570 ×, B, D and F at 6750 ×.

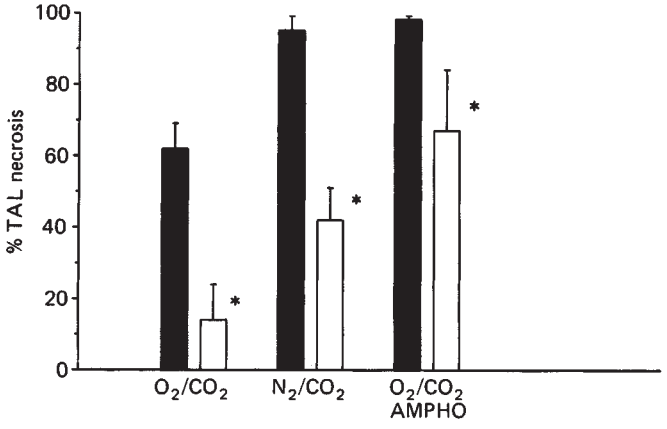

Fig. 2. *Effect of acidosis on extent of hypoxic TAL injury.* The percent of TAL tubules with severe fragmentation damage was assessed after 60 min of oxygenated (O_2/CO_2), non-oxygenated (N_2/CO_2) or amphotericin perfusion. Perfusions were at pH 7.4 (closed bars) or pH 7.0 (open bars). *Indicates $P < 0.05$ for pH 7.0 vs. pH 7.4 for each experimental condition.

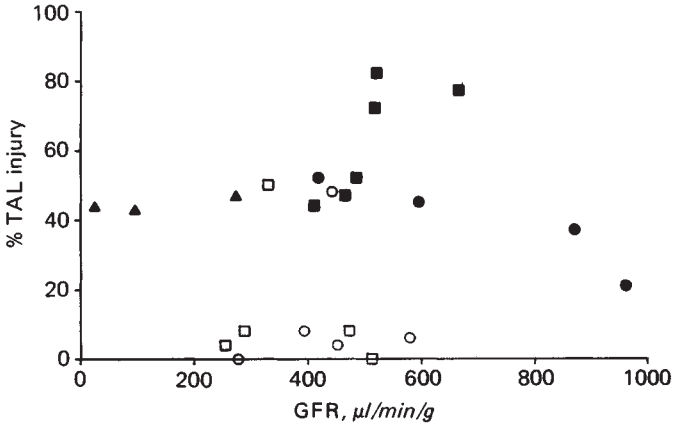

Fig. 3. *Relationship of TAL injury to GFR.* Each point represents one experiment with conditions as follows:

pH 7.4	pH 7.0
(■) Control conditions	(□) Control conditions
(●) High perfusion pressure	(○) High perfusion pressure
(▲) Hyperoncotic albumin	

7.4 the cellular damage seems at least in part related to exacerbation of cellular hypoxia. This results from the combination of vascular effects which decrease O_2 delivery and enhancement of O_2 demand in tubule cells from Na,K-ATPase activity stimulated by the drug's ionophore effect [17]. At pH 7.0 amphotericin was not as cytotoxic as at pH 7.4, even though

oxygen delivery was reduced equivalently at both levels of perfusate pH.

A number of cellular responses to acidosis have been described which could be relevant in the mechanism of protection from hypoxia at low extra-cellular pH. These include "stabilization of membranes" [4, 19] and decreased calcium influx [6, 7, 20]. Since Na,K-ATPase is a pH dependent enzyme [21] and it has been reported that a moderate acidosis will inhibit Na,K-ATPase activity in vitro [22, 23], it is also possible that acidosis directly reduces TAL transport activity. Thus, by a mechanism similar to that presumed operative in the protection afforded by ouabain or furosemide in this model [12, 13], the resultant decreased O_2 demand could account for the cytoprotection observed. Finally, since the TAL necrosis in this model may be a consequence of a mitochondrial, electron flow-dependent mechanism [24–26], it is possible that extracellular acidosis ultimately has effects on mitochondrial function.

In summary, the present study shows that in the isolated perfused kidney extracellular acidosis has divergent effects. Whole kidney function is significantly impaired while there is protection of TAL from hypoxic necrosis. The mechanism of the cytoprotection does not appear to be related to preventing TAL hypoxia either by improving O_2 delivery or by indirectly inhibiting TAL transport by altering solute delivery. The mechanism by which acidosis interrupts the cellular pathogenesis of hypoxic necrosis in this model remains speculative but the effects of acidosis on plasma membrane structure, calcium ion homeostasis, TAL transport activity and mitochondrial metabolism appear to warrant further study.

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References

1. Williamson JR, Schaffer SW, Ford C, Safer B: Contribution of tissue acidosis to ischemic injury in the perfused rat heart. *Circulation* 53:1, 1976
2. Chan L, Ledingham JG, Clarke J, Ross BD: The importance of pH in acute renal failure, in *Acute Renal Failure*, edited by Eliahou HE, London, John Libbey, 1982
3. Pentilla A, Trump BF: Extracellular acidosis protects Ehrlich ascites tumor cells and rat renal cortex against anoxic injury. *Science* 185:277, 1974
4. Penttila A, Glaumann H, Trump BF: Studies on the modification of the cellular response to injury. IV. Protective effect of extracellular acidosis against anoxia, thermal, and *p*-chloromercuribenzene sulfonic acid treatment of isolated rat liver cells. *Life Sci* 18:1419, 1976
5. Bonventre JV, Cheung JY: Effects of metabolic acidosis on viability of cells exposed to anoxia. *Am J Physiol* 249:C149, 1985
6. Weinberg JM: Oxygen deprivation-induced injury to isolated rabbit kidney tubules. *J Clin Invest* 76:1193–1208, 1985
7. Burnier M, Burke TJ, Shanley PF, Schrier RW: Effect of extracellular acidosis in enhanced CA influx in anoxic renal proximal tubules. (abstract) *Kidney Int* 29:299A, 1986
8. Bing OHL, Brooks WW, Messer JV: Heart muscle viability following hypoxia: Protective effect of acidosis. *Science* 180:1297, 1973
9. Brezis M, Rowen S, Silva P, Epstein FH: Renal ischemia: A new perspective. *Kidney Int* 26:375–383, 1984
10. Alcorn D, Kerry RE, Ross BD, Ryan GB, Tange JD: Selective distal nephron damage during isolated kidney perfusion. *Kidney Int* 19:638–647, 1981
11. Brezis M, Rosen S, Silva P, Epstein FH: Selective vulnerability of the medullary thick ascending limb to anoxia in the isolated perfused rat kidney. *J Clin Invest* 73:182–190, 1984
12. Brezis M, Rosen S, Silva P, Epstein FH: Transport activity modifies thick ascending limb damage in the isolated perfused kidney. *Kidney Int* 25:65–72, 1984
13. Brezis M, Rosen S, Spokes K, Silva P, Epstein FH: Transport-dependent anoxic cell injury in the isolated perfused rat kidney. *Am J Pathol* 116:327–341, 1984
14. Ross B, Leaf A, Silva P, Epstein FH: Na-K-ATPase in sodium transport by the perfused rat kidney. *Am J Physiol* 226(3):624–629, 1974
15. Block MH, Ruegg P, Karr M: A glycol methacrylate embedding technique emphasizing cost containment, ultrarapid processing and adaptability to a variety of stains. *Lab Med* 13:290–298, 1982
16. Shanley PF, Brezis M, Spokes K, Silva P, Epstein FH, Rosen S: Hypoxic injury in the proximal tubule of the isolated perfused rat kidney. *Kidney Int* 29:1021–1032, 1986
17. Brezis M, Rosen S, Silva P, Spokes K, Epstein FH: Polyene toxicity in renal medulla: Injury mediated by transport activity. *Science* 224:66–68, 1984
18. Shanley PF, Brezis M, Spokes K, Silva P, Epstein FH, Rosen S: Transport-dependent cell injury in the S_3 segment of the proximal tubule. *Kidney Int* 29:1033–1037, 1986
19. Bell ML, Lazarus HM, Herman AH, Egdahl RH, Rutenburg AM: pH dependent changes in cell membrane stability (35251). *Proc Soc Exp Biol Med* 136:298–300, 1971
20. Studer RK, Borle AB: Effect of pH on the calcium metabolism of isolated rat kidney cells. *J Membr Biol* 48:325–341, 1979
21. Huang WH, Askari A: Regulation of $(Na^+ + K^+)$-ATPase by inorganic phosphate: pH dependence and physiological implications. *Biochem Biophys Res Commun* 123:438–443, 1984
22. Eaton DC, Hamilton KL, Johnson KE: Intracellular acidosis blocks the basolateral Na-K pump in rabbit urinary bladder. *Am J Physiol* 247:F946–F954, 1984
23. Wingo CS: Effect of acidosis on chloride transport in the cortical thick ascending limb of Henle perfused *in vitro*. *J Clin Invest* 78: 1324–1330, 1986
24. Brezis M, Rosen S, Shanley P, Spokes K, Silva P, Epstein FH: Electron flow dependent cell damage: An unrecognized element of anoxic injury? (abstract) *Clin Res* 32(2):442A, 1984
25. Brezis M, Rosen S, Silva P, Spokes K, Epstein FH: Mitochondrial activity: A possible determinant of anoxic injury in renal medulla. *Experientia* 42:570–572, 1986
26. Shanley PF, Johnson GC: Evidence for mitochondrial (mito) electron transport dependent necrosis in the thick ascending limb of Henle (TAL). *Abstracts of the 21st Annual Meeting of the American Society of Nephrology*, Dec. 1988